mitochondrial membrane. The mechanism by which VDAC controls ion flow is important to understanding cellular metabolic processes. It remains an unresolved problem despite publication of the structure of VDAC's open conformation. According to the first gating model proposed by Marco Colombini's group, gating occurs concurrently with large structural rearrangements, and there exist multiple conformations of closed states. These closed states have not been examined by diffraction or NMR, and thus obtaining structural information on VDAC under conditions that promote closure are valuable to unraveling the gating mechanism.

Accordingly we have used magic angle spinning NMR (MAS NMR) and electrophysiological measurements to study recombinant human VDAC1 in lipid bilayers under extreme pH conditions that have been shown to perturb VDAC gating and structure. Detergent-solubilzed membrane proteins are often not amenable to solution NMR studies at extreme pH conditions; thus MAS NMR is uniquely positioned to study the structure and dynamics of closed conformations of VDAC in lipid bilayers. At pH 4 and lower we observe changes in chemical shifts and peak intensities for some, but not all, residues in VDAC's N-terminus, and for residues not in the voltage-sensing domain. Changes in chemical shifts for some residues in the N-terminus are reversed when the pH is raised from low to neutral values. Electrophysiological experiments with VDAC reconstituted into a planar lipid membrane confirmed that VDAC functions properly at pH values as low as 3.0. Furthermore, low pH enhances voltage-gating, and this pH effect is fully reversible. We discuss the implications of our observations and evaluate possible gating mechanisms.

47-Plat

A structural Comparison of the N-Terminal Segment of the Apelin Receptor in Various Membrane Mimetics

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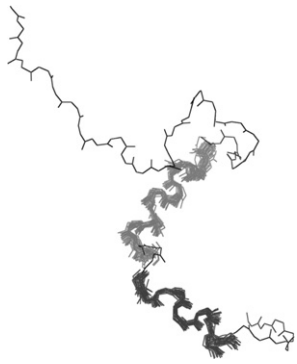
Membrane mimetics such as micelles, bicelles or bilayers provide an essential tool for studying membrane proteins and their fragments. In this study we present the structure of the N-terminal tail and first transmembrane segment of the apelin receptor (human APJ residues 1-55, APJ55). Using sodium dodecylsuphate (SDS), dodecylphosphocholine (DPC) and 1-palmitoyl-2-hydroxy-sn-glycero-3-phospho-(1′-rac-glycerol) (LPPG) alongside phospholipid bicelles, the structure of APJ55 is compared by both circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy. Far-UV CD spectroscopy demonstarates that APJ55 adopts a largely helical structure in each environment. Through 1H-15N HSQC NMR experiments, APJ55 is observed to be in similar though distinct conformations in SDS, DPC and LPPG micelles. The high-resolution structure of APJ55 in SDS micelles was solved and consists of a helix-kink-helix motif in the micelle-spanning transmembrane region. The solution-exposed N-terminal tail has several regions of converged structure. The structure and topology of APJ55 in SDS micelles is critically compared to those in DPC and LPPG micelles, and to low-resolution structural data in phospholipid bicelles, using solution- and solid-state NMR. Overall, this study provides important insight into the consistency of structures generated through different membrane mimetics.



48-Plat

Investigation of Structural Changes Upon Ligand Binding of the Methylated Neuropeptide Y Receptor Type 2

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G protein-coupled receptors (GPCRs) are membrane spanning proteins, which represent very important drug targets. For any pharmacological interference, detailed knowledge about the structure and dynamics of the molecules are essential, in particular those structural changes are relevant that occur upon ligand binding to the receptor. Here, we have studied the neuropeptide Y receptor type 2 (Y2R), which belongs to the class A of GPCRs and has a wide variety in function.

We are able to produce large amounts (35 mg/l minimal media) of the receptor in a prokaryotic expression system as inclusion bodies. These protein aggregates were isolated, solubilized in SDS-micelles and purified. Subsequently, the receptor was refolded into its functional state [1]. Our aim is to investigate ligand-specific conformational changes of the receptor by NMR spectroscopy. Therefore, we used the reductive methylation of lysine residues to introduce 13C-methyl groups [2]. Due to their favorable relaxation properties, these methyl groups allow for sensitive NMR-measurements. We detected 1H-13C HSQC NMR spectra, which provide some resolved NMR signals of the respective methyl groups. Chemical shift changes of some signals are observable, which are induced by ligand binding. These chemical shift changes could be related to alterations of salt bridges or ring current effects that occur due to structural changes induced by ligand binding. We are currently working on the assignment of the residues using site-directed mutagenesis.

A very astonishing side effect is that the methylated receptor shows a dramatic increase in the stability.

[1] P. Schmidt et al. (2009). Biotechnol. Prog. 25(6):1732-9.
[2] M.P. Bokoch et al. (2010). Nature 463(7277):108-12.

49-Plat

Comparison Analysis of Primary Ligand Binding Sites in Seven-Helical Membrane Proteins

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Seven-helix transmembrane proteins, including the G-protein coupled receptors, mediate a broad range of fundamental cellular activities through binding to a wide range of ligands. Understanding the structural basis for the ligand-binding selectivity of these proteins is of significance to their structure-based drug design. Comparison analysis of proteins' ligand binding sites provides a useful way to study their structure-activity relationships. Various computational methods have been developed for the binding site comparison of soluble proteins. In this work, we applied this approach to the analysis of the primary ligand-binding sites of 92 seven-helix transmembrane proteins. Results of the studies confirmed that the binding site of bacterial rhodopsins is indeed different from all G-protein coupled receptors. In the latter group, further comparison of the binding sites indicated a group of residues that could be responsible for ligand-binding selectivity and important for structure-based drug design. Further, unexpected binding site dissimilarities were observed among adrenergic and adenosine receptors, suggesting that the percentage of the overall sequence identity between a target protein and a template protein alone is not sufficient for selecting the best template for homology modeling of seven-helix membrane proteins. These results provided novel insight into the structural basis of ligand-binding selectivity of seven-helix membrane proteins and are of practical use to the computational modeling of these proteins.

PLATFORM B: Emerging Single Molecule Techniques I

50-Plat

Visualizing the Dynamics of DNA Polymerase Exchange Through Simultaneous Single-Molecule Measurements of Replisome Composition and Function

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A complete understanding of the molecular mechanisms underlying the functioning of large, multiprotein complexes requires experimental tools capable of simultaneously visualizing molecular architecture and enzymatic activity in real time. We developed a novel single-molecule assay that combines the flow-stretching of individual DNA molecules to measure the activity of the DNA-replication machinery with the visualization of fluorescently labeled DNA polymerases at the replication fork. By correlating polymerase stoichiometry with DNA synthesis of T7 bacteriophage replisomes, we are able to quantitatively describe the mechanism of polymerase exchange. We find that even at relatively modest polymerase concentration (~2 nM), soluble polymerases are recruited to an actively synthesizing replisome, dramatically increasing local polymerase concentration. These excess polymerases remain passively associated with the replisome through electrostatic interactions with the T7 helicase for ~50 seconds until a stochastic and transient dissociation of the synthesizing polymerase from the primer-template allows for a polymerase exchange event to occur.